PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A23L 2/00 — A2

(11) International Publication Number: **WO 99/38394**

(43) International Publication Date: 5 August 1999 (05.08.99)

(21) International Application Number: PCT/US99/02026

(22) International Filing Date: 29 January 1999 (29.01.99)

(30) Priority Data:
09/016,474 30 January 1998 (30.01.98) US

(71) Applicant *(for all designated States except US)*: FLOW INTERNATIONAL CORPORATION [US/US]; 23500 64th Avenue South, Kent, WA 98032 (US).

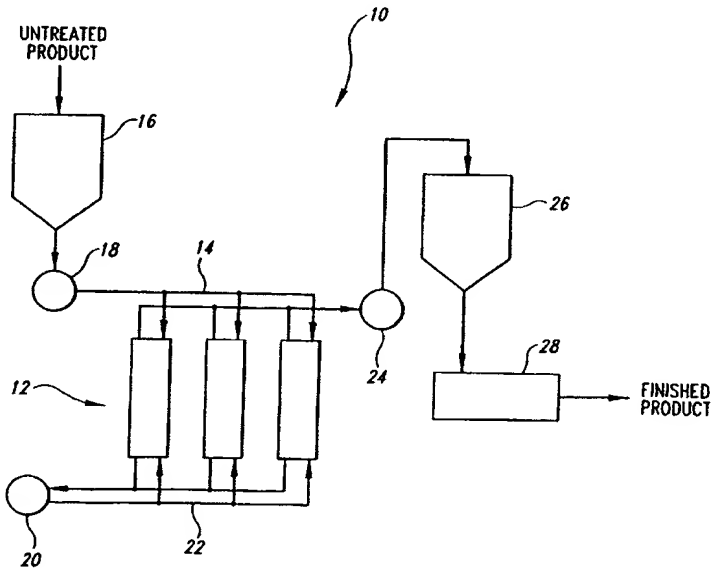
(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: RAGHUBEER, Errol, V. [US/US]; 36932 Second Avenue Southwest, Federal Way, WA 98003 (US). TING, Edmund, Y. [US/US]; 23642 123rd Place Southeast, Kent, WA 98031 (US).

(74) Agents: LOOP, Thomas, E. et al.; Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: METHOD FOR ULTRA HIGH PRESSURE INACTIVATION OF MICROORGANISMS IN JUICE PRODUCTS



(57) Abstract

Methods for preparing juices having an extended shelf-life without the need for pasteurization are disclosed. Such methods employ ultra high pressure (UHP) to substantially inactivate microorganisms associated with juices. The resulting juice products retain many of the preferred fresh juice characteristics such as taste, nutrition, texture and color, characteristics that may be destroyed or diminished by thermal processing or pasteurization.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

METHOD FOR ULTRA HIGH PRESSURE INACTIVATION OF MICROORGANISMS IN JUICE PRODUCTS

TECHNICAL FIELD

The present invention is generally directed to the preparation of juices having an extended shelf-life without the need for thermal pasteurization. More particularly, the present invention is directed to methods for inactivating microorganisms in juices through the application of ultra high pressures, as well as to juice products manufactured by these methods.

BACKGROUND OF THE INVENTION

Foods and beverages have traditionally been preserved to prolong their shelf-life through the use of chemicals and thermal treatment. The U.S. Food and Drug Administration (FDA), for example, currently mandates thermal processing (*i.e.*, pasteurization) for many commercial food and beverage products as a way of increasing public safety against the potential harmful effects of food borne diseases. The FDA has long recognized thermal processing as an effective means for inactivating microorganisms, such as harmful pathogens, that may exist in many unprocessed food and beverage products. If left unchecked, these microorganisms can not only cause premature food and beverage spoilage, but can also result in serious health problems for humans.

In particular, the FDA has been particularly concerned with minimizing the hazards associated with fresh juice products. This concerns stems from several recent (*i.e.*, October 1996) outbreaks of food borne illness caused by the consumption of apple juice and cider contaminated with harmful *Escherichia coli* O157:H7, the same bacterium associated with the much publicized nation-wide recall of hamburger patties during the summer of 1996. Ironically, apple juice was once considered low-risk in terms of food safety, because its low pH was thought to inhibit the proliferation of harmful bacteria. This thinking has changed, however, as a result of the reported sixty-six illnesses and one death caused by the consumption of contaminated apple juice

distributed by a Pacific Northwest (United States) beverage processor. In that outbreak, unpasteurized juices were found to be contaminated with dangerous levels of *Escherichia coli* O157:H7, resulting in the recall of the contaminated apple juice throughout nine western states of the United States and British Columbia, Canada. Later that same month in Connecticut, incidences of *Escherichia coli* O157:H7 infection were attributed to the consumption of unpasteurized apple cider.

Growing concern over the presence of *Escherichia coli* O157:H7 and other pathogenic organisms in juice products has prompted the FDA to propose a comprehensive program aimed at minimizing the hazards associated with fresh fruit and vegetable beverages. The proposal, published in the Federal Register on August 28, 1997, calls for a mandatory Hazard Analysis of Critical Control Points (HACCP) program, the establishment of industry-wide good manufacturing practices, mandatory pasteurization, increased collection of samples for laboratory analysis, and warning labels on many juice products. The FDA indicated in its proposed program, however, that when a beverage processor delivers a 5-log reduction in *Escherichia coli* O157:H7 to the product, the warning label will not be required. The FDA has intimated that pasteurization is the only validated process that can achieve this standard. *See* Federal Register, August 28, 1997, which is hereby incorporated herein by reference.

The FDA push for pasteurization runs contrary to the traditional preference of many consumers for unpasteurized fruit and vegetable juices. In particular, pasteurization is perceived by many consumers as destroying or diminishing many preferred fresh juice characteristics like taste, nutrition, texture, and color.

Accordingly, there is a need in the art for a new method for producing a safe juice product, without the loss of the preferred fresh juice characteristics.

SUMMARY OF THE INVENTION

In brief, the present invention provides methods for inactivating microorganisms associated with a juice. In one embodiment, the method includes the following steps: introducing the juice into an internal isolator chamber of a processing system, wherein the processing system includes an ultra high pressure pump for pressurizing the isolator chamber, the processing system being capable of controllably

maintaining an ultra high pressure within the isolator chamber for a selected period of time; pressurizing the isolator chamber to a pressure of at least 50,000 psi; maintaining the juice within the isolator chamber for a period of time ranging from 10 to 480 seconds to substantially inactivate the microorganisms; depressurizing the isolator chamber to substantially atmospheric pressure; and, discharging the juice from the isolator chamber into an associated filling station.

In another embodiment, the method includes placing the juice within a food processor capable of controllably maintaining an ultra high pressure for a selected period of time; and, subjecting the juice to a pressure of at least 50,000 psi for a period of time ranging from 10 to 480 seconds to substantially inactivate microorganisms contained within the juice.

The present invention is also directed to juice products having inactivated microorganisms, wherein the juice is manufactured by the steps of placing the juice within a food processor capable of controllably maintaining an ultra high pressure for a selected period of time; and, subjecting the juice to a pressure of at least 50,000 psi for a period of time ranging from 10 to 480 seconds.

The present invention is also directed to an ultra high pressure beverage processing system for inactivating microorganism associated with a juice. The ultra high pressure beverage processing includes a first storage tank for holding the juice before ultra high pressure beverage processing; a second storage tank for holding the juice after ultra high pressure beverage processing; at least one isolator adapted to hold the juice at an ultra high pressure for a selected period of time; a first piping system interconnecting the first storage tank to at least one of the isolator and to the second storage tank, wherein the first piping system includes at least one first valve adapted to regulate the juice flow into at least one of the isolator; at least one ultra high pressure pump adapted to pressurize the juice to an ultra high pressure; and, a second piping system interconnecting at least one of the ultra high pressure pump to at least one of the isolator, wherein the second piping system includes at least one second valve adapted to regulate a pressurizing fluid flow into at least one of the isolator.

These and other aspects of this invention will be apparent to one skilled in the art upon reference to following detailed description, drawing and associated experimental data.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a process flow diagram depicting a representative process for continuous operation of an embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention provides methods for inactivating microorganisms associated with a juice. In general, the methods of this invention employ ultra high pressure (UHP) to substantially inactivate microorganisms associated with an unpasteurized juice. Put differently, this invention discloses methods for producing a juice product that has a prolonged shelf-life by subjecting the juice to an ultra high pressure for a period of time sufficient to inactivate microorganisms that may be contained within the juice. The resulting juice product typically retains many of the preferred fresh juice characteristics such as taste, nutrition, texture, and color, characteristics that may be destroyed or diminished by pasteurization.

In one embodiment, an unpasteurized or raw juice is initially introduced into an internal isolator chamber of an ultra high pressure food processing system. One such food processing system is commercially available from Flow International Corporation (Kent, Washington); however, any pressure chamber capable of maintaining an ultra high pressure for a selected period of time may be adapted to work with the methods of the present invention. The food processing system available from Flow International includes an ultra high pressure (UHP) pump designed to controllably pressurize the internal isolator chamber. The UHP pump is capable of pressurizing the juice within the isolator chamber to pressures ranging from 50,000 to over 100,000 pounds per square inch (psi). As used herein, the term "juice" includes fresh squeezed juices, juice blends, juice concentrates, fortified juices, and ciders, as well as any other fluid extract derived from any variety of plant, such as fruits or vegetables, or any combination thereof. Juices typically have pH's ranging from 3 to 4.5. Apple juice and orange juice are two preferred juices that may be used in accordance with the methods disclosed herein.

Before being introduced into the isolator chamber, the juice may, however, be hermetically sealed within a flexible container, such as a heavy-duty plastic bag. The flexible container facilitates processing during batch operation of the food

processing system. Alternatively, the juice may be pumped directly into the isolator chamber during continuous or semi-continuous operation of the food processing system. After the juice has been introduced into the isolator chamber, the UHP pump is activated in order to pressurize the isolator chamber to an ultra high pressure of at least 50,000 psi. The ultra high pressure is then maintained for a selected period of time. As used herein, the term "ultra high pressure" refers to any pressure above 40,000 psi. Preferably, the pressure within the isolator chamber is maintained at about 80,000 psi for approximately 60 seconds. The isolator chamber is then depressurized to substantially atmospheric pressure, and the juice is discharged into an associated filling station such as a stainless steel vat. The process may be repeated for as many times as necessary to meet production demands.

For high volume commercial applications, continuous operation of the food processing system may be preferable. As illustrated in Figure 1, continuous production of the food processing system 10 may be achieved by using multiple isolators 12 interconnected by a common first piping system 14. In this embodiment and similar to the operation of a four stroke multi-cylinder engine, each isolator 12 may alternate between filling, pressurizing, holding, depressurizing, and emptying. Accordingly, continuous production of the food processing system 10 initially involves introducing an untreated or raw juice into a first storage tank 16. The raw juice is then sequentially pumped by pump 18 into each of the multiple isolators 12. A series of valves and regulators (not shown) in the first piping system 14 may control the timing, volume, and flow rate of juice into each isolator 12. After a single isolator 12 has been charged with juice, that isolator is then pressurized by an associated ultra high pressure pump 20 to an ultra high pressure of at least 50,000 psi. It should be noted that a separate piping system 22 interconnects the ultra high pressure pump 20 to the multiple isolators 12, and a series of valves and regulators (not shown) may control the timing, volume, and flow rate of pressurizing fluid (*e.g.*, water) into each isolator 12. The ultra high pressure is then held for a selected period of time; preferably, the pressure is maintained at about 80,000 psi for approximately 60 seconds.

The juice is then depressurized to substantially atmospheric pressure and pumped by pump 24 into an associated second storage or surge tank 26. The juice is then transferred into an associated filling machine 28. The filling machine 28 is used to

fill appropriate containers, such plastic and/or glass bottles, with the finished inactivated juice product.

It should be further noted that the first and second storage tanks 16, 18, filling machine 30, as well as the associated ancillary equipment are all standard pieces of equipment in the food and beverage processing industry and are therefore readily available to those skilled in the art.

The above-identified methods are beneficial in that they substantially inactivate microorganisms associated with raw juices without the need for thermal processing or pasteurization. As used herein, the term "microorganism" refers to any living organism of microscopic or ultramicroscopic size and includes germs, viruses, microbes, molds, yeast, bacteria, as well as all known pathogens. Furthermore, as used herein, the term "inactivate" means to destroy or to substantially impede the proliferation of microorganisms. Thus, a method for inactivating microorganisms associated with a juice refers to a method that results in a commercially sterile juice product, wherein "commercially sterile" has its standard meaning in the art and as understood by the FDA. In the context of fruit and vegetable juices, a method shown to achieve a greater than 5-log reduction in *Escherichia coli* O157:H7 is considered to result in a commercially sterile juice product. Accordingly, a 5-log reduction in *Escherichia coli* O157:H7 in a juice product is considered to inactivate this pathogen.

It should be noted that the methods of the present invention may preferably be performed at room temperature (RT) or approximately 68°F, and with batch or continuous operation of the food processing system. The methods of the present invention may, however, be performed at higher temperatures, and it is believed that temperatures above 100°F will reduce the amount of time and/or pressure needed to inactivate the microorganisms. In addition to juices, other products that may benefit from the disclosed ultra high pressure methods include jams, jellies, sauces, salsas, soups, wines, yogurt, and pharmaceuticals. Thus, any product that may harmed by high temperature treatment or pasteurization will likely benefit from ultra high pressure processing. Moreover, processing costs may be as low as few cents a pound depending upon the food or beverage and the goals of processing.

In order to better illustrate the effectiveness of the novel methods disclosed herein, several experiments were conducted to repeatedly demonstrate at least a 5 log reduction in the level of pathogens, namely *Escherichia coli* O157:H7 and *Listeria monocytogenes*, associated with unpasteurized juices (*i.e.*, apple juice and orange juice). The specific experimental materials, methods, and results are more fully set forth below.

A. MATERIALS AND METHODS

1. Bacterial Cultures and Inoculation Levels

a. Preconditioning of Cultures.

For purposes of experimentation, several strains of microorganisms (*i.e.*, *Escherichia coli* O157:H7 and *Listeria monocytogenes*) were obtained (*see* Table 1) and inoculated into sterile apple and/or orange juice in a manner that is appreciated and well understood by those skilled in the art. Specifically, all strains of microorganisms tested in the study, except the FDA 6 strain from the Pacific Northwest (United States) juice outbreak, were inoculated at least twice into sterile apple and/or orange juice and recovered on MacConkey Sorbitol agar. The recovered strains were biochemically confirmed and stored on Trypticase Soy Agar slants. Inoculation levels were determined on 3M Coliform Petri Film for *E. coli* and Modified Oxford Agar for *L. monocytogenes*.

b. *Escherichia coli* O157:H7:

Eight strains of *E. coli* 0157:H7 (*see* Table 1) were used as the inoculum. Each strain was grown separately overnight (ca. 18 hrs) in brain heart infusion broth (BHI, DIFCO). Fifteen milliliters of each were combined and used as the inoculum for the apple juice and orange juice samples. The FDA 6 strain was tested independently. The inoculated level with the eight strain mixture was 2.8×10^6 CFU/ml of juice. The FDA 6 strain was inoculated at a level of 1.4×10^7 CFU/ml.

c. ***Listeria monocytogenes*:**

Two strains of *L. monocytogenes* (*see* Table 1) were grown independently in BHI overnight. 50 milliliters of each were combined and inoculated into apple juice and orange juice at a level of 2.3×10^6 CFU/ml.

5

2. Enumeration Procedures

a. Enumeration of *E. coli* O157:H7

Enumeration was done by two methods: The 3-tube Most Probable Number (MPN) in EC medium containing 1% sodium pyruvate and with 3M coliform 10 Petrifilm. Tubes and Petrifilms were checked at 48 hours and allowed to incubate for five days at 37°C.

b. Enumeration of *Listeria monocytogenes:*

Similarly, the 3-tube MPN method in Listeria selective medium (LEB) 15 and direct plating on MOX were used for enumeration. All LEB (MPN) tubes were streaked onto MOX after incubation for four days at 30°C. MOX plates were incubated for three days at 35°C.

TABLE 1. MICROORGANISMS USED FOR INOCULATED JUICE UHP STUDY

20

Lab. ID	Description	Source	Provider
***Escherichia coli* 0157:H7**			
FDA 1	Seattle 13A24; SLT 1,2	Meat Loaf	Steve Weagant, FDA
FDA 2	Seattle 13A46; SLT 1,2	Hamburger	Steve Weagant, FDA
FDA 3	Seattle 13A29; SLT 1,2	Dry Salami	Steve Weagant, FDA
FDA 4	Sea 6318; SLT 1,2	Jack-In-The Box Hamburger	Steve Weagant, FDA
FDA 5	Sea 6458; SLT 1,2	Jack-In-The Box Hamburger	Steve Weagant, FDA
FDA 6	Sea 13B88; SLT 1,2	Odwalla Unpasteurized Juice	Steve Weagant, FDA
NFFEc 1	ATCC 43889, SLT 2	Stool, HUS Patient	ATCC
NFFEc 2	ATCC 43895, SLT 1,2	Raw Hamburger	ATCC
NFFEc 3	R2292	85% Partially Defatted Beef	Lab Isolate
Listeria monocytogenes			
NFFLm 1	V7	Chocolate Milk	Carlos Abeyta, FDA
NFFLm 2	R1950	Pasteurized Salted Egg Yolk	Lab Isolate

B. EXPERIMENTAL RESULTS

1. *E. coli* in Apple Juice (6 hrs. after UHP Treatment)

a. 60,000 psi

For the eight strain inoculum, there were no significant effects on the levels of *E. coli* at 60,000 psi for 30 seconds (*see* Table 2). There was, however, a 3-log decrease in the levels of *E. coli* after 60 seconds, and after 180 seconds 9.3 MPN/ml were detected.

For the FDA 6 strain (not shown in Table 2), the response to 60,000 psi was different. There were 21,000 and 2,400 cells recovered for 30 and 60 seconds of treatment, respectively. No cells were detected at 120 and 180 seconds of treatment.

b. 80,000 psi

The effects of 80,000 psi UHP on the survival of the eight strain mix of *E. coli* were significantly different from the effects of 60,000 psi UHP (*see* Table 2). At 80,000 psi for 30 seconds of treatment there was a 4-log reduction from 2.8×10^6 CFU/ml to 460 MPN/ml. No cells were detected at 80,000 psi for 60, 120 and 180 seconds of treatment.

For the FDA 6 strain (not shown in Table 2), no cells were detected for all treatment times at 80,000 psi.

2. *E. coli* in Orange Juice

The response of the eight strain mix of *E. coli* in orange juice was similar to that in apple juice (*see* Table 2). The decrease in viable cells for the four treatment times at 60,000 psi was approximately the same as in apple juice. As with the 80,000 psi treatment in apple juice, no cells were detected for 60, 120 and 180 seconds of treatment.

The response of the FDA 6 strain was different than the eight strain mixture (not shown in Table 2). In orange juice, survival was detected at all treatment times at 60,000 psi and at the 30 seconds of treatment at 80,000 psi. No cells were detected at 80,000 psi for 60, 120 and 180 seconds of treatment.

3. *E. coli* Recovery Results for UHP Treated Samples

Recovery tests were conducted after one week and again after one month of storage for UHP treated samples stored at room and refrigerated temperatures (*see* Table 2). No survivors were detected for any of the treatment times at 60,000 and 80,000 psi, including treatments of 30 seconds. It appears that the cells were stressed during UHP treatment and did not recover in the low pH environment. Microbiological analyses on inoculated untreated samples supported this conclusion. After one week of storage, relatively high levels of *E. coli* cells were detected in these samples with greater levels enumerated in the refrigerated samples. After three weeks, the level of *E. coli* in untreated inoculated samples was still 1.1×10^3 MPN/ml. The recovery results for the FDA 6 strain were not available.

4. *L. monocytogenes* in Apple and Orange Juice (6 hrs. after UHP treatment)

Listeria monocytogenes appears to be more sensitive to UHP than *E. coli* O157:H7 (*see* Table 3). There was a 4-log decrease in apple juice at 30 seconds of treatment at 60,000 psi. No survival of *L. monocytogenes* was detected for any of the other treatments at both 60,000 and 80,000 psi.

5. *L. monocytogenes* Recovery Results for UHP Treated Samples

No survivors were detected after 1 week and after 1 month of storage for UHP treated samples stored at room and refrigerated temperatures (*see* Table 3). However, in the untreated inoculated samples stored at refrigeration, relatively high numbers of *L. monocytogenes* were still surviving after 6 weeks. As with the *E. coli* untreated inoculated samples, there was a significantly higher level of survival in samples stored at refrigerated temperature compared to the room temperature samples. This difference in survival in different temperatures has been well reported, particularly for low pH foods.

TABLE 2. *E. COLI* O157:H7 IN APPLE JUICE AND ORANGE JUICE

UHP treatment: 60,000 & 80,000 psi
Products: Pasteurized shelf stable TreeTop® 100% Apple Juice **pH**=3.80
Pasteurized shelf stable Orange Tap® 100% Orange Juice **pH**=3.84
Baseline Count (Before Inoculation):
Apple Juice: APC = ND, Yeast = ND, Coliform = <0.3 MPN/ml, *E. coli* = Neg/25 ml, *Listeria* Neg/25 ml
Orange Juice: APC = 10, yeast = ND, Coliform = <0.3 MPN/ml, *E. coli* = Neg/25 ml, *Listeria* Neg/25 ml
Inoculum: *E. coli* 0157:H7 (8 strains mix) @ 2.8 x 10^6/CFU/ml
6 hrs. after inoculation (8 strain mix): 7.8 x 10^5 CFU/ml (Apple Juice); 7.3 x 10^5 CFU/ml (Orange Juice)
6 hrs. after inoculation (FDA 6 strain): 2.9 x 10^7 CFU/ml (Apple Juice); 3.1 x 10^7 CFU/ml (Orange Juice)
ND (non detected); **MPN** (Most Probable Number: 3-tube);
UHP (Ultra High Pressure)
CFU (Colony Forming Units); **APC** (Aerobic Plate Count)

Sample #	UHP (seconds)	*E. coli* MPN/ml (6 hrs. after UPH)	*E. coli* MPN/ml: After 1 week @ Room Temp.	*E. coli* MPN/ml: After 1 week @ Refrigeration	*E. coli* After 1 month @ Room Temp.	*E. coli* After 1 month @ Refrigeration
APPLE JUICE: Controls		Room Temp.				
1 **No UHP/No inoc**		**Neg/25 ml**	**Neg/25 ml**	**Neg/25 ml**	Not tested	Not tested
2 **No UHP/ Inoculated**		**780,000**	**43,000***	**700,000***	<0.3	**4.3**
APPLE JUICE @ 60,000 psi						
3	30	>11,000	<10*	<10*	<0.3	<0.3
4	60	>1,100	<0.3	<0.3	<0.3	<0.3
5	120	110	<0.3	<0.3	<0.3	<0.3
6	180	9.3	<0.3	<0.3	<0.3	<0.3
APPLE JUICE @ 80,000 psi						
7	30	460	<0.3	<0.3	<0.3	<0.3
8	60	<0.3	<0.3	<0.3	<0.3	<0.3
9	120	<0.3	<0.3	<0.3	<0.3	<0.3
10	180	<0.3	<0.3	<0.3	<0.3	<0.3
ORANGE JUICE: Controls						
11 **No UHP/No inoc**		**Neg/25 ml**	**Neg/25 ml**	**Neg/25 ml**	Not tested	Not tested
12 **No UHP/ Inoculated**		**730,000**	**80**	**680,000**	<0.3	<0.3
ORANGE JUICE @ 60,000 psi						
13	30	>1,100	<10	<10	<0.3	<0.3
14	60	>1,100	<0.3	<0.3	<0.3	<0.3
15	120	110	<0.3	<0.3	<0.3	<0.3
16	180	7.5	<0.3	<0.3	<0.3	<0.3
ORANGE JUICE @ 80,000 psi						
17	30	460	<0.3	<0.3	<0.3	<0.3
18	60	<0.3	<0.3	<0.3	<0.3	<0.3
19	120	<0.3	<0.3	<0.3	<0.3	<0.3
20	180	<0.3	<0.3	<0.3	<0.3	<0.3

* Plate count. All others 3-tube MPN in mEC medium.

TABLE 3. *LISTERIA MONOCYTOGENES* IN APPLE JUICE AND ORANGE JUICE

UHP treatment: 60,000 & 80,000 psi
Products: Pasteurized shelf stable TreeTop® 100% Apple Juice **pH**=3.80
Pasteurized shelf stable Orange Tap® 100% Orange Juice **pH**=3.84
Baseline Count (Before Inoculation):
Apple Juice: APC = ND, Yeast = ND, Coliform = <0.3 MPN/ml, *E. coli* = Neg/25 ml, *Listeria* Neg/25 ml
Orange Juice: APC = 10, yeast = ND, Coliform = <0.3 MPN/ml, *E. coli* = Neg/25 ml, *Listeria* Neg/25 ml
Inoculum: *Listeria monocytogenes* (2 strains mix) @ 2.3 x 10^6/CFU/ml
6 hrs. after inoculation: 4.2 x 10^5 CFU/ml (Apple Juice); 1.2 x 10^5 CFU/ml (Orange Juice)
ND (non detected); **MPN** (Most Probable Number: 3-tube);
UHP (Ultra High Pressure)
CFU (Colony Forming Units); **APC** (Aerobic Plate Count)

Sample #	UHP (seconds)	*L. mono-cytogenes* (6 hrs. after UPH)	*L. mono*: After 1 week @ Room Temp.	*L. mono*: After 1 week @ Refrigeration	*L. mono*: After 1 month @ Room Temp.	*L. mono*: After 1 month @ Refrigeration
APPLE JUICE: Controls						
31 **No UHP/No inoc**		**Neg/25 ml**	**Neg/25 ml**	**Neg/25 ml**	Not tested	Not tested
32 **No UHP/ Inoculated**		**420,000**	**100**	**170,000**	<0.3	**27,000**
APPLE JUICE @ 60,000 psi						
33	30	ND	<10	<10	<0.3	<0.3
34	60	ND	<0.3	<0.3	<0.3	<0.3
35	120	ND	<0.3	<0.3	<0.3	<0.3
36	180	ND	<0.3	<0.3	<0.3	<0.3
APPLE JUICE @ 80,000 psi						
37	30	ND	<0.3	<0.3	<0.3	<0.3
38	60	ND	<0.3	<0.3	<0.3	<0.3
39	120	ND	<0.3	<0.3	<0.3	<0.3
40	180	ND	<0.3	<0.3	<0.3	<0.3
ORANGE JUICE: Controls						
21 **No UHP/No inoc**		**Neg/25 ml**	**Neg/25 ml**	**Neg/25 ml**	Not tested	Not tested
22 **No UHP/ Inoculated**		**120,000**	**<10**	**120,000**	<0.3	**9.3**
ORANGE JUICE @ 60,000 psi						
23	30	640	<10*	<10*	<0.3	<0.3
24	60	ND**	<0.3	<0.3	<0.3	<0.3
25	120	ND	<0.3	<0.3	<0.3	<0.3
26	180	ND	<0.3	<0.3	<0.3	<0.3
ORANGE JUICE @ 80,000 psi						
27	30	ND	<0.3	<0.3	<0.3	<0.3
28	60	ND	<0.3	<0.3	<0.3	<0.3
29	120	ND	<0.3	<0.3	<0.3	<0.3
30	180	ND	<0.3	<0.3	<0.3	<0.3

* Plate counts (all others 3-tube MPN: enrichment & plating)
ND** None Detected (no growth on plates)

Finally, comparative data for inoculated samples that were not UHP treated are shown in Tables 4 and 5.

TABLE 4. COMPARATIVE DATA OF 8-STRAIN MIX

APPLE JUICE: E. coli O157:H7. 8-Strain Mix			ORANGE JUICE: E. coli O157:H7. 8-Strain Mix	
Days	RT	Refrig	RT	Refrig
0	2800000	NA	2800000	NA
0.25	780000	NA	730000	NA
7	43000	700000	80	680000
10	2.3	420000	1.5	4600
14	<0.3	130000	<0.3	0.9
17	<0.3	45000	<0.3	0.7
19	<0.3	9300	<0.3	0.7
21	<0.3	1100	<0.3	0.4
26	<0.3	150	<0.3	0.4
30	<0.3	4.3	<0.3	<0.3
34	<0.3	4.3	<0.3	<0.3
38	<0.3	2.3	<0.3	<0.3
50	<0.3	0.4	<0.3	<0.3

TABLE 5. COMPARATIVE DATA OF 2-STRAIN MIX

APPLE JUICE: Listeria monocytogenes 2-Strain Mix			ORANGE JUICE: Listeria monocytogenes 2-Strain Mix	
Days	RT	Refrig	RT	Refrig
0	2300000	NA	2800000	NA
0.25	420000	NA	120000	NA
7	100	170000	<10	120000
10	<10	110000	<10	96000
14	<0.3	84000	<0.3	18000
17	<0.3	81000	<0.3	9000
19	<0.3	67000	<0.3	3100
21	<0.3	63000	<0.3	1500
26	<0.3	57000	<0.3	9.3
30	<0.3	27000	<0.3	9.3
34	<0.3	10400	<0.3	0.7
38	<0.3	4500	<0.3	0.7
50	<0.3	1700	<0.3	0.4

Based on the foregoing experimental data, the methods disclosed herein have repeatedly shown a greater than 5-log reduction in *Escherichia coli* O157:H7 and

Listeria monocytogenes associated with a juice, when the juice is subjected to a pressure of at least 80,000 psi for at least 60 seconds. Accordingly, the present invention demonstrates a novel way for inactivating microorganisms associated with a juice without pasteurization.

While the methods of the present invention have been described in the context of the embodiments and the experimental data illustrated and described herein, the invention may be embodied in other specific ways or in other specific forms without departing from its spirit or essential characteristics. Therefore, the described embodiments and experimental data are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are to be embraced within their scope.

CLAIMS

1. A method for inactivating microorganisms associated with a juice, comprising the steps of:

introducing the juice into an internal isolator chamber of a processing system, wherein the processing system includes an ultra high pressure pump for pressurizing the isolator chamber, the processing system being capable of controllably maintaining an ultra high pressure within the isolator chamber for a selected period of time;

pressurizing the isolator chamber to a pressure of at least 50,000 psi;

maintaining the juice within the isolator chamber for a period of time ranging from 10 to 480 seconds to substantially inactivate the microorganisms;

depressurizing the isolator chamber to substantially atmospheric pressure; and

discharging the juice from the isolator chamber into an associated filling station.

2. The method according to claim 1 wherein the microorganisms are selected from yeast, molds, and bacteria.

3. The method according to claim 1 wherein the microorganisms are pathogens.

4. The method according to claim 3 wherein the pathogens are selected from *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

5. The method according to claim 1 wherein the juice is a fruit juice.

6. The method according to claim 1 wherein the juice is selected from apple juice and orange juice.

7. The method according to claim 6 wherein the pressure is at least 80,000 psi.

8. The method according to claim 7 wherein the period of time ranges from 60 to 180 seconds.

9. The method according to claim 8 wherein the juice has a temperature of approximately 68°F.

10. The method according to claim 9 wherein the processing system is operated in a continuous or batch mode.

11. A method for producing a juice having a prolonged shelf-life, comprising:

placing the juice within a food processor capable of controllably maintaining an ultra high pressure for a selected period of time; and

subjecting the juice to a pressure of at least 50,000 psi for a period of time ranging from 10 to 480 seconds to substantially inactivate microorganisms contained within the juice.

12. The method according to claim 11 wherein the microorganisms are selected from yeast, molds, and bacteria.

13. The method according to claim 11 wherein the microorganisms are pathogens.

14. The method according to claim 13 wherein the pathogens are selected from *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

15. The method according to claim 11 wherein the juice is a fruit juice.

16. The method according to claim 11 wherein the juice is selected from apple juice and orange juice.

17. The method according to claim 16 wherein the pressure is at least 80,000 psi.

18. The method according to claim 17 wherein the period of time ranges from 60 to 180 seconds.

19. The method according to claim 18 wherein the juice has a temperature of approximately 68°F.

20. The method according to claim 19 wherein the food processor is operated in a continuous or batch mode.

21. A juice having inactivated microorganisms, wherein the juice is manufactured by the steps comprising of:

placing the juice within a food processor capable of controllably maintaining an ultra high pressure for a selected period of time; and

subjecting the juice to a pressure of at least 50,000 psi for a period of time ranging from 10 to 480 seconds.

22. The juice according to claim 21 wherein the inactivated microorganisms are selected from yeast, molds, and bacteria.

23. The juice according to claim 22 wherein the inactivated microorganisms are pathogens.

24. The juice according to claim 23 wherein the pathogens are selected from *Escherichia coli* O157:H7 and *Listeria monocytogenes.*

25. The juice according to claim 21 wherein the juice is a fruit juice.

26. The juice according to claim 21 wherein the juice is selected from apple juice and orange juice.

27. The juice according to claim 26 wherein the pressure is at least 80,000 psi.

28. The juice according to claim 27 wherein the period of time ranges from 60 to 180 seconds.

29. The juice according to claim 28 wherein the temperature is maintained at approximately 68°F.

30. The juice according to claim 29 wherein the food processor is operated in a continuous or batch mode.

31. A process for manufacturing a commercially sterile juice, comprising the steps of:

introducing the juice into a first storage tank;

pumping the juice through a first piping system into at least one isolator adapted to hold the juice at an ultra high pressure for a selected period of time;

pressurizing the juice with at least one ultra high pressure pump to an ultra high pressure of at least 50,000 psi;

maintaining the juice within the isolator for a period of time ranging from 10 to 480 seconds;

depressurizing the juice to substantially atmospheric pressure;

discharging the juice into a second storage tank; and

filling at least one container with the juice.

32. An ultra high pressure beverage processing system for inactivating microorganisms associated with a juice comprising:

a first storage tank for holding the juice before ultra high pressure beverage processing;

a second storage tank for holding the juice after ultra high pressure beverage processing;

at least one isolator adapted to hold the juice at an ultra high pressure for a selected period of time;

a first piping system interconnecting the first storage tank to at least one of the isolator and to the second storage tank, wherein the first piping system includes at least one first valve adapted to regulate the juice flow into at least one of the isolator;

at least one ultra high pressure pump adapted to pressurize the juice to an ultra high pressure; and

a second piping system interconnecting at least one of the ultra high pressure pump to at least one of the isolator, wherein the second piping system includes at least one second valve adapted to regulate a pressurizing fluid flow into at least one of the isolator.

1/1

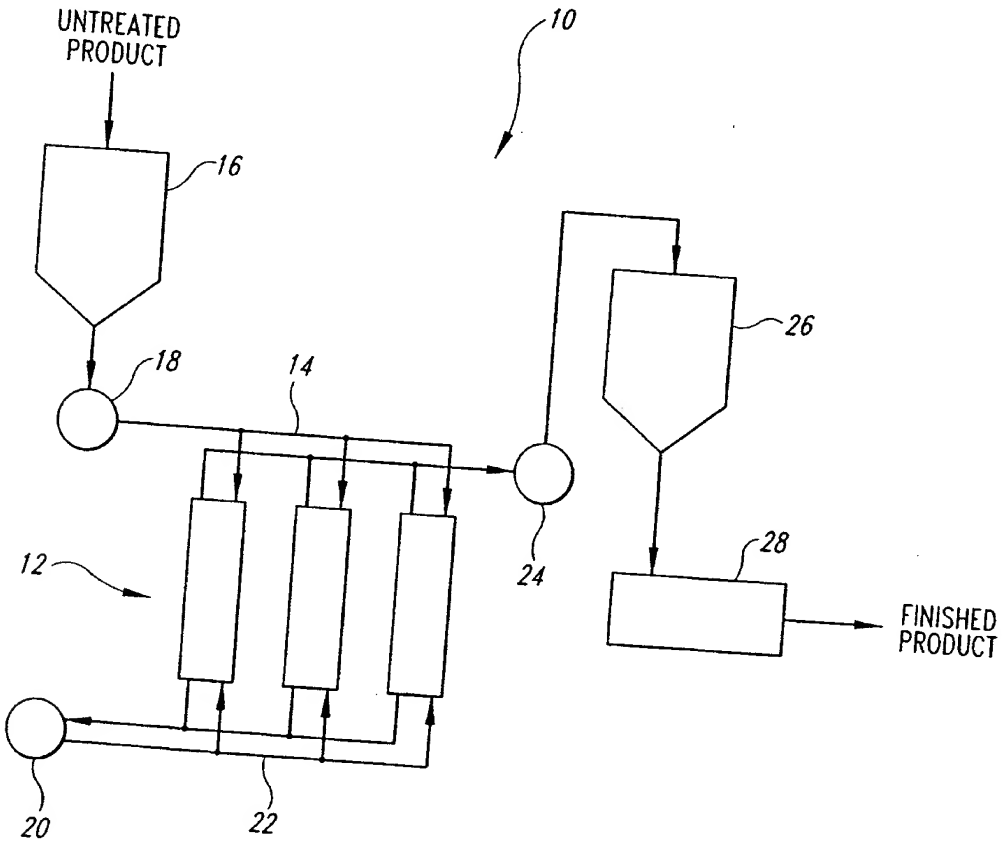

UNTREATED PRODUCT
10
16
18
14
26
12
24
28
FINISHED PRODUCT
20
22

Fig. 1